complexes, now that the complete sets of motor proteins are at hand in many organisms. Finally, in vitro reconstruction experiments and simulations will have to be attempted with the physiologically relevant motor complexes to indicate the extent to which known components can or cannot account for spindle morphogenesis.

An obvious criticism of Nédélec's work is that it represents a "what if," rather than a "what is," approach. Although this is true, the hope is that the "what if" will lead us to the "what is," which is difficult to directly examine in a real spindle. A second, possibly more troubling, criticism is the exclusive emphasis on motor protein-microtubule interactions in spindle morphogenesis. Such a motor-centric view is being challenged by the emerging role of chromosomes in spindle assembly (Karsenti and Vernos, 2001) and by the resuscitation of potential nonmicrotubule structural elements in the spindle (Kapoor and Mitchison, 2001; Walker et al., 2000). However, Nédélec makes an important contribution by showing that a certain theoretical motor configuration is capable of recapitulating an essential aspect of spindle structure in a simplified scenario. These intriguing "virtual" results are sure to stimulate both deeper analysis of spindles and the adoption of similar approaches in other areas of biology.

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Three-Dimensional Structure of a COPII Prebudding Complex

The coat protein complex II (COPII) catalyzes transport vesicle formation from the endoplasmic reticulum. Crystallographic analysis of a Sec23/24-Sar1 prebudding complex of COPII now provides a molecular view of this GTPase-directed coat assembly mechanism.

The COPII coat is composed of the small GTPase Sar1 and two large protein complexes, Sec23/24 and Sec13/31. Coat assembly proceeds in a stepwise manner under the control of Sar1. Activated Sar1-GTP binds to membranes first and recruits the Sec23/24 complex. These Sec23/24-Sar1 prebudding intermediates are then collected by the Sec13/31 complex as subunits polymerize to form coated transport vesicles. Conversion to Sar1p-GDP triggers coat release and recycles COPII subunits (Antonny and Schekman, 2001). As for other GTPases in biology, the nucleotide exchange and hydrolysis rates of Sar1p are governed by extrinsic factors. One of the COPII proteins, Sec23, functions as a GTPase-activating protein (GAP) for Sar1. Moreover, the Sec23 GAP activity toward Sar1 is further stimulated when Sec23/24-Sar1 complexes are gathered by Sec13/31. This mode of GTPase regulation seems to provide COPII with a built-in disassembly program (Antonny et al., 2001)

Sar1 not only controls COPII budding, but also integrates coat assembly with cargo selection. Under conditions that restrict Sar1 to its active GTP-bound form, Sec23/24-Sar1 prebudding intermediates accumulate in complexes with protein cargo to be included in COPII vesicles (Kuehn et al., 1998; Aridor et al., 1998). Based on these findings, current models envisage that membrane-bound Sar1-GTP reversibly links vesicle cargo to prebudding complexes and, therefore, to the COPII coat.

Although a hierarchy of events in COPII budding has been established, our molecular view of this process remains rudimentary. More specifically, it is not understood how coat polymerization leads to membrane deformation, how the variety of cargo to be included in COPII vesicles are recognized by coat subunits, or how Sar1 regulates the coat assembly/disassembly stages to execute these tasks. In addressing these questions, structural information has become imperative. Fortunately, a most satisfying atomic level structure of the Sar1-Sec23/24 prebudding complex is now in hand. In the September issue of *Nature*, Bi et al. (2002) present the three-dimensional structures of the Sec23/24 complex and a cocomplex of Sar1-GppNhp (hereafter referred to as Sar1-GTP) bound to Sec23. Only subtle changes occur in the Sec23 subunit when bound to Sec24 or to Sar1-GTP, thus allowing the authors to construct a composite model of the Sec23/24-Sar1 prebudding complex. This new structural information now bears directly on issues related to membrane deformation, cargo recognition, and Sar1 regulation.

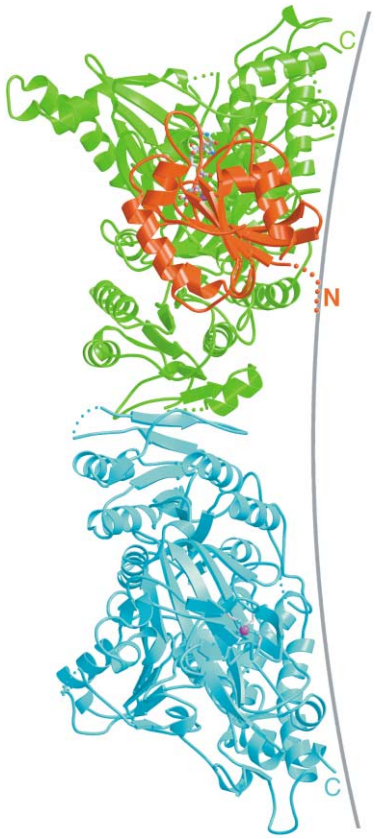
How might the COPII coat sculpt membranes? A side view of the complex (see Figure) reveals an extended "bowtie-shaped" structure with a concave surface that roughly conforms to the surface of a 60 nm vesicle. Basic amino acid residues are enriched on this concave surface in accord with a requirement for acid phospholipids in reconstitution of COPII budding from chemically defined liposomes (Matsuoka et al., 1998). Moreover, this orientation places the hydrophobic N terminus of Sar1-GTP toward the membrane surface, potentially imbedding residues into the bilayer (Huang et al., 2001). The authors point out that this surface curvature would favor membrane deformation when confined by the

Sec13/31 complex and produce uniformly sized vesicles, as has been observed when COPII vesicles are synthesized from ER membranes in the presence of nonhydrolyzable GTP analogs (Matsuoka et al., 1998).

This crystal structure also provides insight into how Sec23/24-Sar1 complexes might recognize cargo. The structural relatedness of the Sec23 and Sec24 proteins are documented, as suggested by primary sequence analysis and high-resolution EM images (Lederkremer et al., 2001; Matsuoka et al., 2001). Sec23 and Sec24 each consist of five distinct domains described as the β barrel, zinc finger, trunk domain, all-helical region, and the gelsolin-like domain. Surfaces from the trunk domains of Sec23 and Sec24 join to form the Sec23/24 heterodimer. Sec23 forms an extensive interface with Sar1-GTP contacting conserved residues contributed by three of the Sec23 domains. However, vast expanses of the Sec23/24 surface remain uncharted, and potential cargo binding sites are described. Given the variety of cargo that must be recognized by the COPII budding machinery, multiple regions of the Sec23/24 protein could be used for cargo selection. Additional studies indicate that distinct Sec24 family members pair with Sec23 and operate in cargo recognition to expand the range of cargo incorporated into prebudding complexes (Antonny and Schekman, 2001).

How is Sar1 regulated during transport vesicle formation? Further inspection of the Sec23-Sar1 interface reveals the mechanism of Sar1 GTPase stimulation. Sec23 furnishes an arginine "finger" to the Sar1 active site, stabilizing the transition state, as has been observed for other GAPs. This explains Sec23 activation of the Sar1 GTPase but does not resolve how prebudding cargo complexes, which contain Sec23 bound to Sar1, are included in polymerized coats before GTP hydrolysis and dissociation occur. Here a cogent model has been presented by Antonny et al. (2001) in which native prebudding complexes posses a relatively slow rate of GTP hydrolysis that is stimulated when gathered into the complete coat by Sec13/31. In other words, Sec23 interaction with Sar1 would proceed in two stages. On the basis of the structural data, one could imagine a first stage in which prebudding Sec23/24-Sar1 complexes position the catalytic arginine residue near the GTPase active site and a second stage in which Sec13/31 binding repositions these active site residues for optimal GTP hydrolysis. The authors speculate that Sec13/31 collects prebudding complexes through associations with the gelsolin-like domains present in both the Sec23 and Sec24 proteins.

How might the Sec23/24-Sar1 complex coordinate its cargo-sorting task with coat assembly? Future studies are likely to focus on how cargo fits into this scenario. Biochemical and structural analyses of defined cargo molecules bound to prebudding complexes should identify molecular contacts. Cargo binding could also influence the Sar1 GTPase rates to extend the lifetime of prebudding intermediates until included into budded vesicles. For an exploration of the effects of cargo, real-time assays that monitor coat assembly/disassembly ates should be informative (Antonny et al., 2001). Indeed, a combination of structural approaches with real-time assays should provide a unique perspective into GTPase-directed control of cargo recognition and coat polymerization.



Structure of the Sec23/24-Sar1 Prebudding Complex

Ribbon representation of the complex with Sec23 colored in green, Sec24 in blue, and Sar1 in red. The gray arc indicates the curvature of a 60 nm vesicle, drawn to scale (adapted from Bi et al. [2002] and kindly provided by J. Goldberg).

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